IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
PETRONIS, *et al.*
Serial No.: 10/598,140
Filed: 9 February 2007

For: CPG-AMPLICON AND ARRAY PROTOCOL

Confirmation No. 1527
Art Unit: 1637
Examiner: Christopher M. Babic
Atty. Dckt: 034263.002
08899871US1

DECLARATION UNDER 37 C.F.R. § 1.132

I, Dr. David I Martin, hereby declare as follows:

1. I am a citizen of the United States of America residing at Berkeley, California, USA.
2. I am a physician by training and experience and received a Medical Doctor Degree from the Medical College of Virgina in 1981; I received post-graduate research training at New york University and Harvard Medical School. .
3. I am currently the Chair of the Center for Genetics and Deputy Director of the Children's Hospital Oakland Research Institute, Oakland, CA, and an Adjunct Member of the Victor Chang Cardiac Research Institute, Sydney, NSW, Australia.
4. I have published extensively in scientific peer-reviewed journals in the subject area of epigenetics, and have more than 20 years of experience in the field.
5. I have reviewed and am familiar with the above-referenced patent application, USSN 10/598,140, as pending (the "patent application") and the claims as currently pending (the "claimed invention").
6. I have no financial or other interest in the claimed invention.
7. In view of my experience and education, I consider myself to be a person skilled in the field of art, i.e. epigenetics, to which the claimed invention belongs.
8. In my opinion, a person of ordinary skill in the art of epigenetics would be a molecular biologist with a doctoral degree and at least 3 to 8 years of experience using molecular genetic methods to analyze epigenetic phenomena and their molecular basis.
9. I have reviewed and I am familiar with:
   a. The Official Action dated 11/30/2009,
   b. Yan et al. (J. Nutr. 2002 Aug; 132 (8 Suppl):2430S-2434S),
   c. Huang (U.S. Patent No. 6,605,432),
   d. Chotai et al. (J. Med. Genet. 1998 Jun; 35(6):472-5), and
   e. Dean (U.S. Patent No. 6,617,137).
10. It is my understanding that the claimed invention is directed to a method of analyzing the unmethylated fraction, i.e. the unmethylated fragments, of one or more nucleotide sequences.

a. It is also my understanding that the inventors of the claimed invention discovered that examination of the unmethylated fraction of one or more nucleotide sequences can be significantly more informative than examination of the methylated fraction as explained at paragraph [0094] of the patent application as filed and as published on page 532 in the attached peer-reviewed scientific article Schumacher et al. (Nucleic Acids Research, 2006, 34(2):528-542).

b. As set forth at paragraph [0094] of the patent application and page 532 of Schumacher et al., analysis of the unmethylated fraction in and around the COMT gene results in over 400 informative fragments whereas analysis of the hypermethylated fraction results in only 6 informative fragments. This is shown diagrammatically in Figure 1 of Exhibit A which is attached herewith. The COMT gene is linked to various neurobehavioral disorders.

c. The profile of the plurality of the unmethylated fragments may be analyzed. For example, see the scatter plots provided in the patent application, and

d. Figure 2 of Exhibit A which shows the methylation patterns in post-mortem brains of controls compared to a patient with late onset Alzheimer's disease. As shown in Figure 2, the arrows indicate that the predisposing gene PSEN1 is hypomethylated in the Alzheimer disease patient. This small but significant methylation shift is detectable and measurable by analysis of the unmethylated fraction but this difference would not be detectable using the methylated fraction of the genome.

11. The Examiner correctly recognizes that neither Yan nor Huang teach the successive digestion of a nucleic acid sample with a methylation-sensitive restriction enzyme followed by a methylation-specific restriction enzyme. Thus, I understand that the Examiner cites Chotai as the motivation for modifying Yan and/or Huang to result in an unmethylated fraction further analysis. I understand that the Examiner cites Dean as a supportive disclosure for genome amplification.

12. Contrary to the Examiner's assertion, in my opinion, one of ordinary skill in the art would not have been motivated to combine and/or modify the disclosures of Yan, Huang, Chotai, and Dean in order to obtain an unmethylated fraction, i.e. a plurality of unmethylated fragments, as provided by the claimed invention. In particular:

a. Yan is directed to analysis of the hypermethylated fraction of nucleotide sequences. Thus, Yan removes hypomethylated and unmethylated fragments. Nowhere does Yan teach or suggest that the unmethylated fragments should be analyzed.

b. Likewise, Huang is directed to analysis of the hypermethylated fraction of nucleotide sequences. Thus, Huang removes hypomethylated and unmethylated fragments. Nowhere, does Huang teach or suggest that the unmethylated fragments should be retained for analysis.

c. Chotai is directed to the analysis of the methylation state of a single genetic locus, the SNRPN locus, in order to distinguish Prader-Willi syndrome (PWS) from Angelman syndrome (AS). As set forth in Figure 1 of Chotai, unmethylated SNRPN is indicated of PWS and methylated SNRPN is indicative of AS. Chotai deals with a specific situation in which a locus is subject to parental imprinting, meaning that it may normally be found in either a methylated or an unmethylated state. It does not

mention anything about any other genetic locus or loci and their methylation states; in fact it conveys the impression that there is no utility to looking further than the locus in question. At the time of the invention it was well understood that imprinted loci normally are found in both a methylated and an unmethylated state, but imprinted loci were known to be a special case and not representative of the genome as a whole; nothing in Chotai can be taken to suggest that examination of loci not subject to imprinting could be productive. Furthermore, nowhere does Chotai teach or suggest that a plurality of unmethylated fragments obtained from one or more nucleotide sequences will likely provide information about whether a subject suffers from PWS or AS.

d. Dean does not mention the methylation status of any DNA sequence anywhere in the entire document.

Since the documents cited by the Examiner, alone or in combination, do not teach or suggest that study of unmethylated DNA will be productive, or that a plurality of unmethylated fragments will provide useful information, in my opinion one of ordinary skill in the art would not have been motivated to modify and/or combine the documents in order to achieve the unmethylated fragments according to the claimed invention.

13. Conventional wisdom prior to the claimed invention was that only methylated and hypermethylated DNA fragments provided useful information, such that those skilled in the art at the time of the invention would have been taught by experts in the field only the enrichment and analysis of the methylated fraction of DNA, and taught away from enriching the unmethylated fraction of DNA for analysis.

14. In my opinion, one of ordinary skill in the art would not, at the time of the invention, have had any reason to believe that the unmethylated fraction of a nucleotide sequence would provide significantly more informative fragments than the methylated fraction: thinking in the field was directed at analysis of methylated DNA, because it had been shown that aberrant hypermethylation of specific loci was linked to disease, and it was known that the majority of CpG sites in genomic DNA are methylated rather than unmethylated.

15. In view of the conventional wisdom at the time of the invention, and the fact that the majority of CpG sites in genomic DNA are methylated rather than unmethylated, I believe that a person skilled in the art would have found it unexpected and surprising that unmethylated fractions obtained from one or more nucleotide sequences can provide significantly more informative fragments than hypermethylated fractions, as exemplified by the analysis of and around the COMT gene. I know of nothing that would have taught the skilled person that analysis of the unmethylated fraction would be productive.

16. Therefore, it is also my opinion that the claimed invention provides superior and unexpected results which are not taught or suggested by Yan, Huang, Chotai, and Dean, either alone or in combination. Yan and Huang teach only the analysis of methylated DNA. Chotai teaches a method to distinguish the methylated from the unmethylated state of an imprinted gene and, by failing to discuss at all the use of such a method in non-imprinted loci, teaches away from the idea that analysis of unmethylated DNA would be productive.

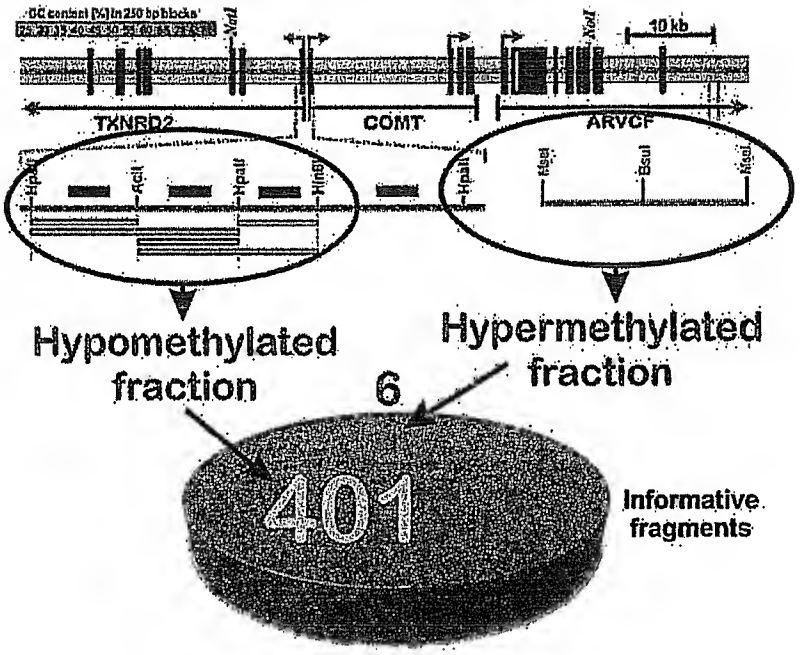
17. I hereby declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true; and further that

these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Signed this 14 day of May, 2010.

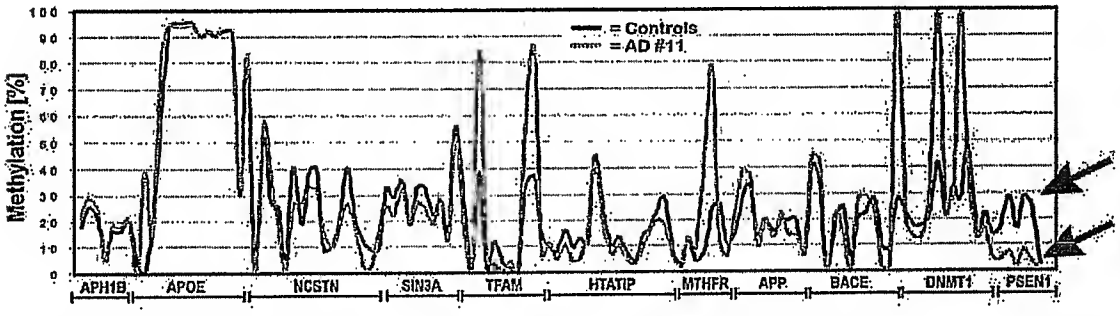
Dr. David I Martin

Figure 1



Genomic region around the COMT gene that is linked to various neurobehavioral disorders. By using the unmethylated fraction of the genome, this genomic region produces over 400 informative fragment, whereas the old method of using the hypermethylated fraction produces only 6 such informative fragments.

Figure 2



Methylation pattern in post-mortem brains of controls compared with a patient twith late-onset Alzheimer disease. The arrows indicate that the predisposing gene PSEN1 is hypomethylated in the AD patient. This small but significant methylation shift would not be detectable using the methylated fraction of the genome, however using the unmethylated fraction it is measurable.